Low-Cost Biocatalyst for Acceleration of Energy Efficient CO_2 Capture

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Advanced Research Projects Agency • ENERGY

Forward-looking statements

These slides and the accompanying oral presentation contain forward-looking statements that involve risks and uncertainties. These statements relate to future events regarding our carbon capture program and involve known and unknown risks, uncertainties and other factors that could cause our actual results, levels of activity, performance or achievement to differ materially from those expressed or implied by these forward-looking statements. Forward-looking statements include all statements that are not historical facts. In some cases, you can identify forward-looking statements by terms such as “may,” “will,” “should,” “could,” “would”, “expects,” “plans,” “anticipates,” “believes,” “estimates,” “projects,” “predicts,” “potential,” or the negative of these terms, and similar expressions and comparable terminology intended to identify forward-looking statements. These forward-looking statements represent our estimates and assumptions only as of the date hereof, and, except as required by law, we undertake no obligation to update or revise publicly any forward-looking statements, whether as a result of new information, future events or otherwise.

Other factors that could materially affect actual results, levels of activity, performance or achievements can be found in Codexis’ Annual Report on Form 10-K dated Feb 20, 2011, including under the caption “Risk Factors.” If any of these risks or uncertainties materialize, or if our underlying assumptions prove to be incorrect, actual results, levels of activity, performance or achievement may vary significantly from what we projected.

About Codexis, Inc.

- World leader in creating ‘super enzymes’ through *Directed Evolution*
- 100’s of Directed Evolution patents
- Presidential Green Chemistry Awardee 2006 and 2010
- Partner with Shell in biofuels
- Collaborating with Chemtex to produce Bio-based detergent alcohols
- ~350 employees California, Hungary and Singapore
- NASDAQ (CDXS)
- Revenues >$100 million for 2010

CO_2 Solution / Codexis IP

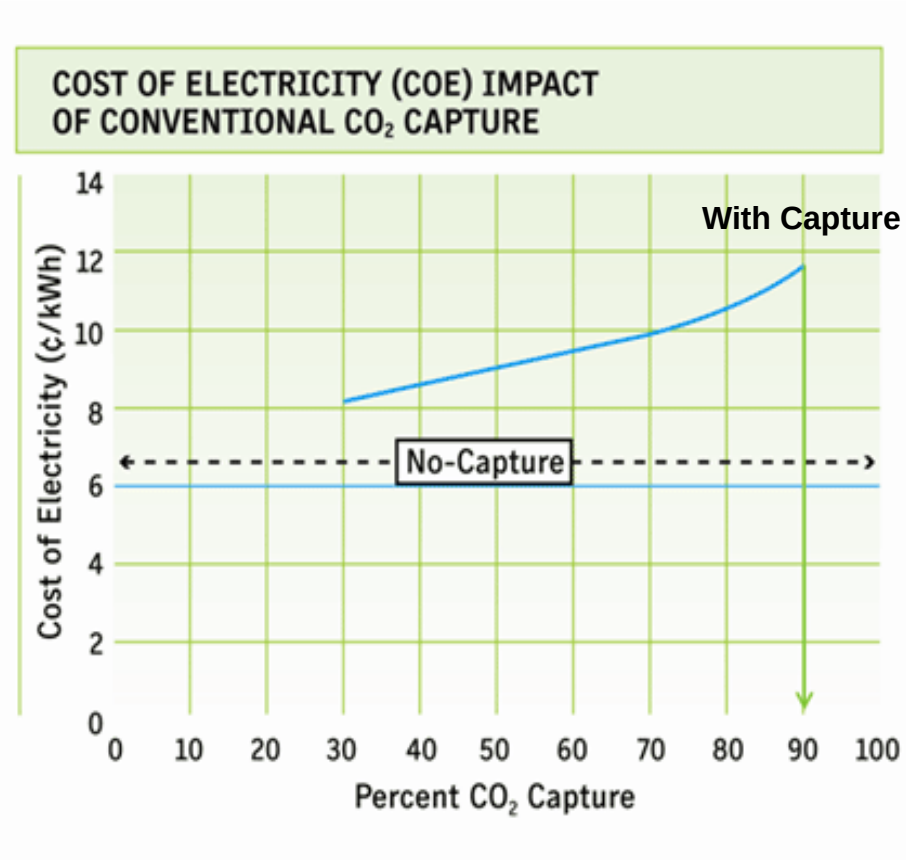
- CO_2 Solution holds a number of issued patents for use of carbonic anhydrase (CA) for carbon capture
  - Enzyme-solvent formulations
  - Processes
  - Sector applications
- Complements Codexis IP portfolio in enzyme evolution and optimized carbonic anhydrases

Selected CO_2 Solution Patents

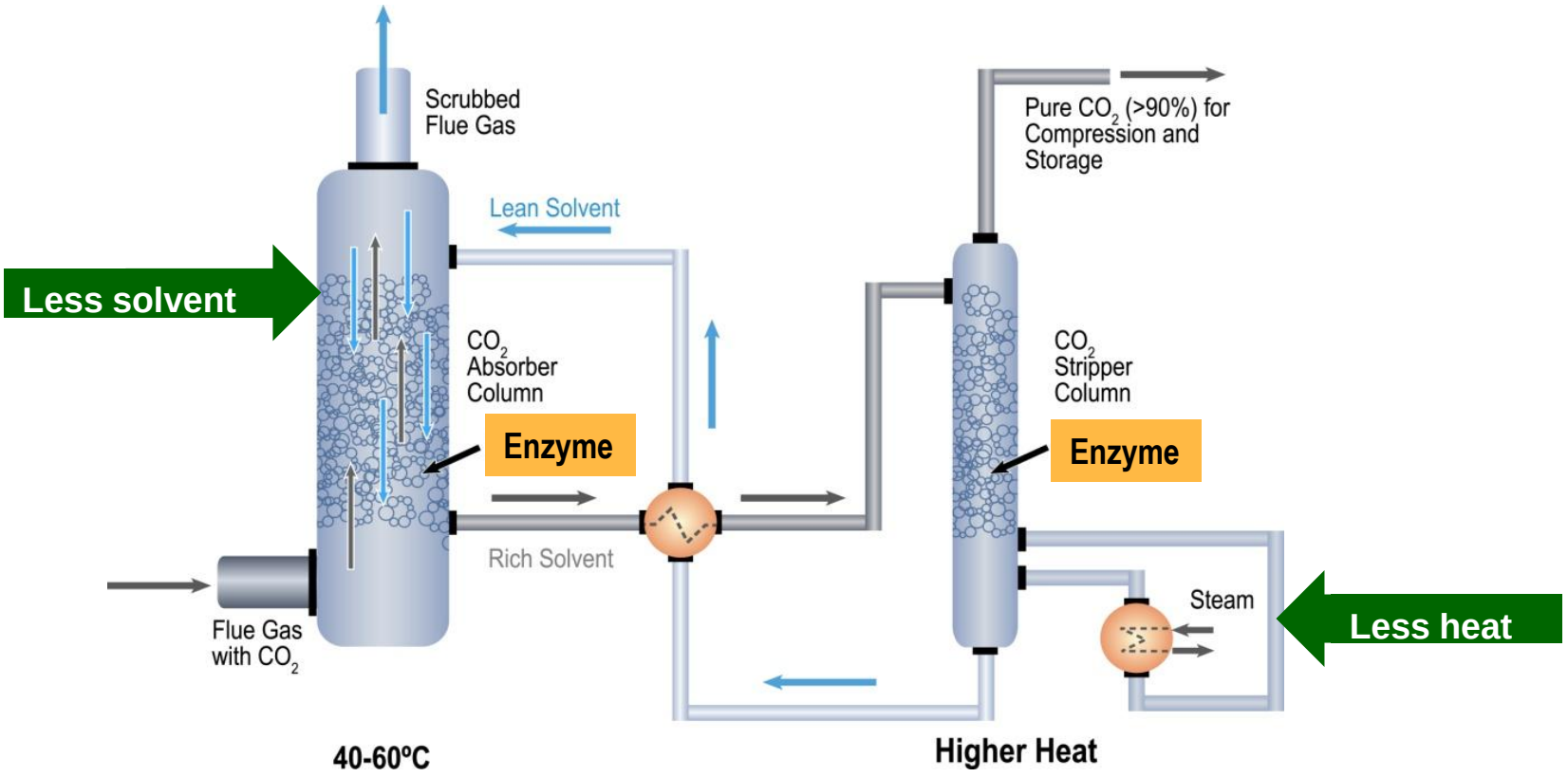
Patent #	Area of Carbonic Anhydrase (CA) CO_2 Capture Application
US 7,740,689	Amine solvents
US 7,596,952	Power plants
US 7,176,017	Triphasic reactor
US 6,524,843	Packed column system
US 6,908,507	Cement production
US 7,521,217	Thermally stable CA variants
US 7,514,056	Air fractionation / oxygen production
US 61/231038	CA on micro-particles
US 61/231037	Carbonate solvents
US 61/231039	Amino acid solvents

Current CO_2 Capture methods too costly

- Existing Solvent Technologies
  - Benefit: Proven, available
  - Challenges
    - Parasitic energy loss high
    - ~$60-80 / ton CO_2 removal cost
    - COE nearly doubles with capture
    - Complex, high CapEx
    - Low ΔH_{desorb} solvents have slow absorption kinetics



Enable energy efficient solvents with enzymes

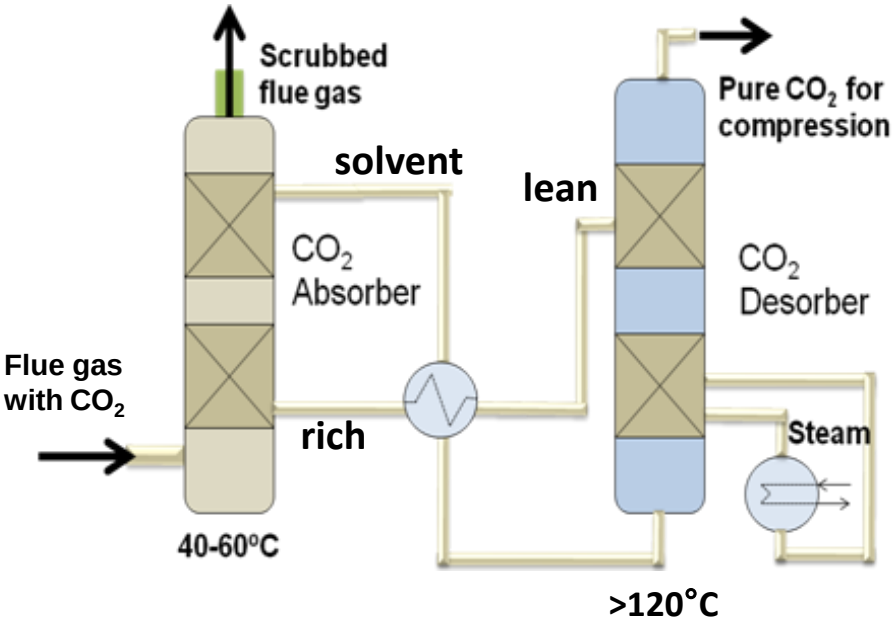


- Custom enzymes allow use of energy efficient solvents.
- Enzyme + solvent requires much less heat to release CO_2.
- Potential to lower increased COE from 80% to $\leq$35%.

Targets

CA

$$CO_2 + H_2O \leftrightarrows HCO_3^- + H^+$$

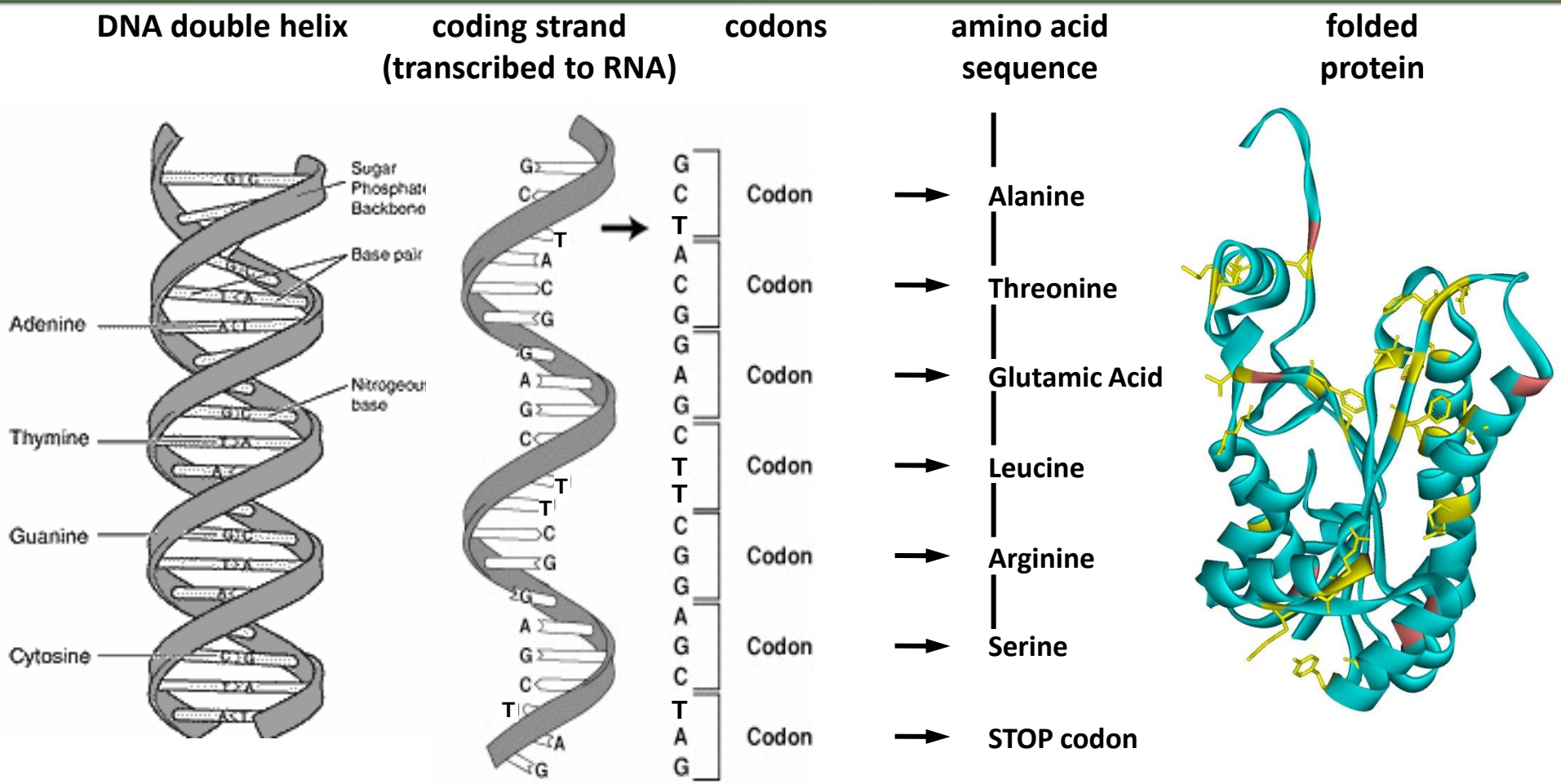


Performance Targets:

- ✓Enzyme: Carbonic Anhydrase (CA)
- ✓Activity & stability in MDEA
  - 50 vol% = 4.2M MDEA
- Thermostability (≥120°C),
- ✓ pH from >11 to 8,
- ✓Soluble Enzyme
- Stability to degradants, flue gas components etc.,
- Economical production of biocatalyst

N-methyldiethanolamine = MDEA

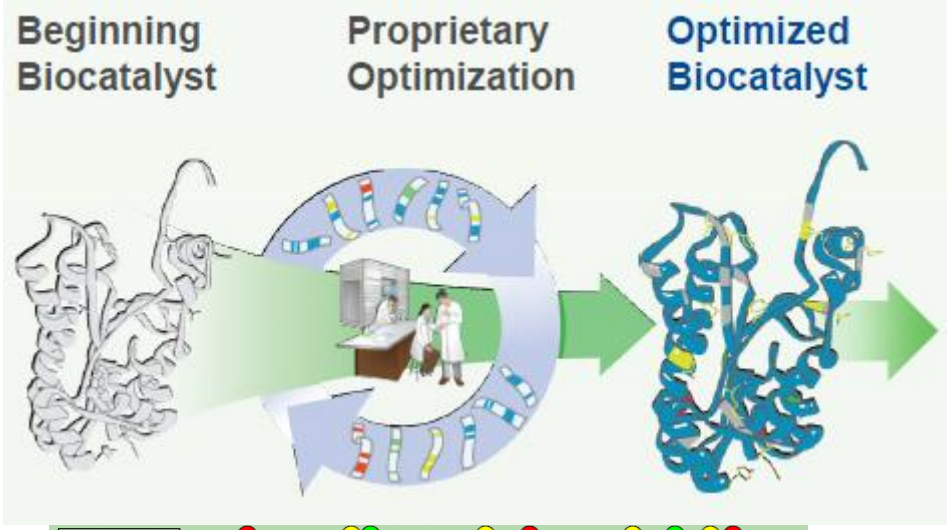
Genes (DNA) Code for Proteins

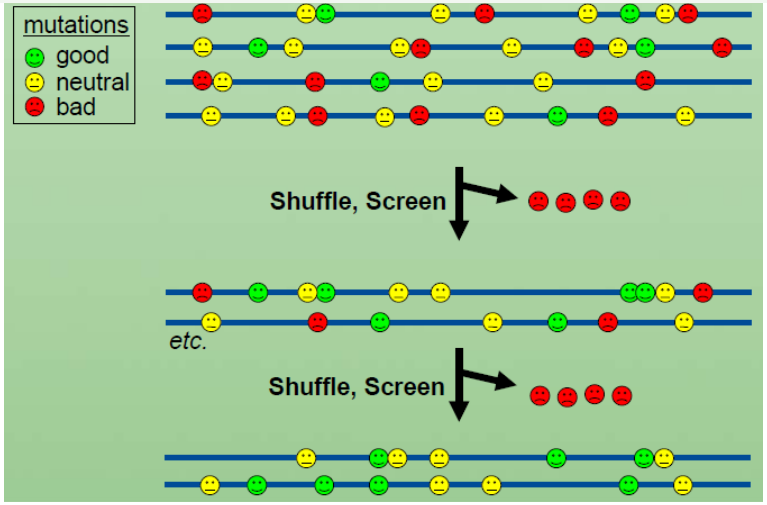


Protein engineering is the recoding of DNA to change (mutate) the amino acids at positions in the sequence to improve the protein's function compared to the wild-type protein.

Directed Evolution Technology

- Codexis technology to design and manufacture enzymes which vastly outperform native enzymes
- Screening identifies new enzymes with beneficial mutations and without detrimental ones
- Validated by success in pharma and biofuels

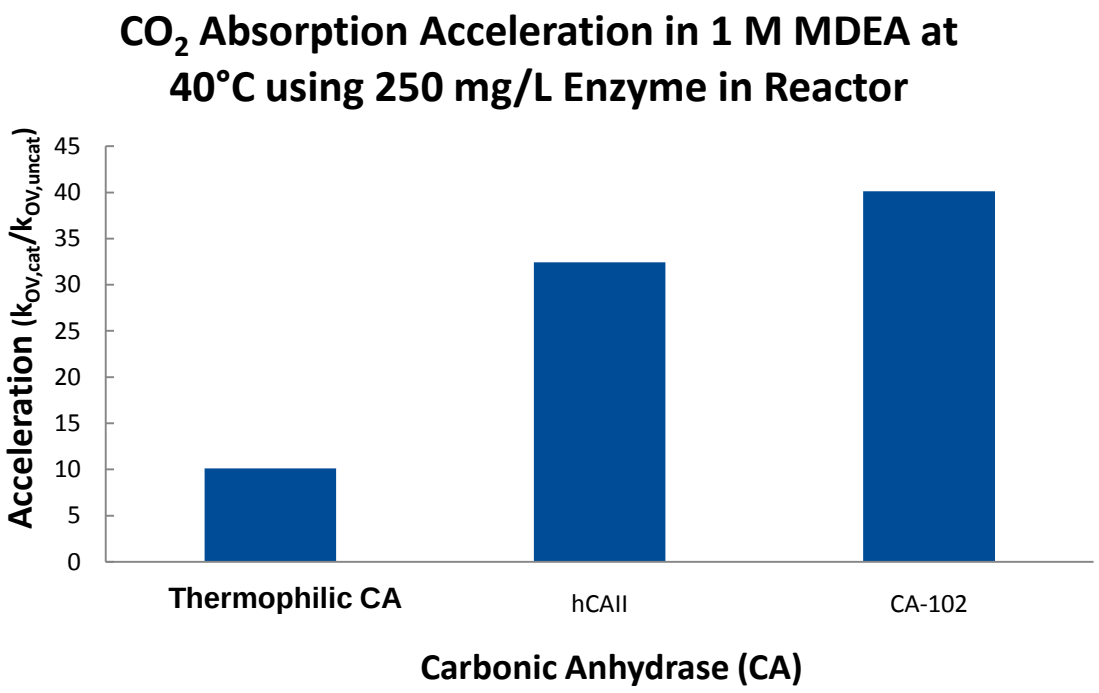




Selecting the Best CA for MDEA

Selection Criteria:

1. High activity in MDEA.
2. High thermo- and solvent-stability.
3. Can be produced economically.



Thermophilic CA:

- Very thermostable.
- Low activity and stability in high MDEA concentrations.

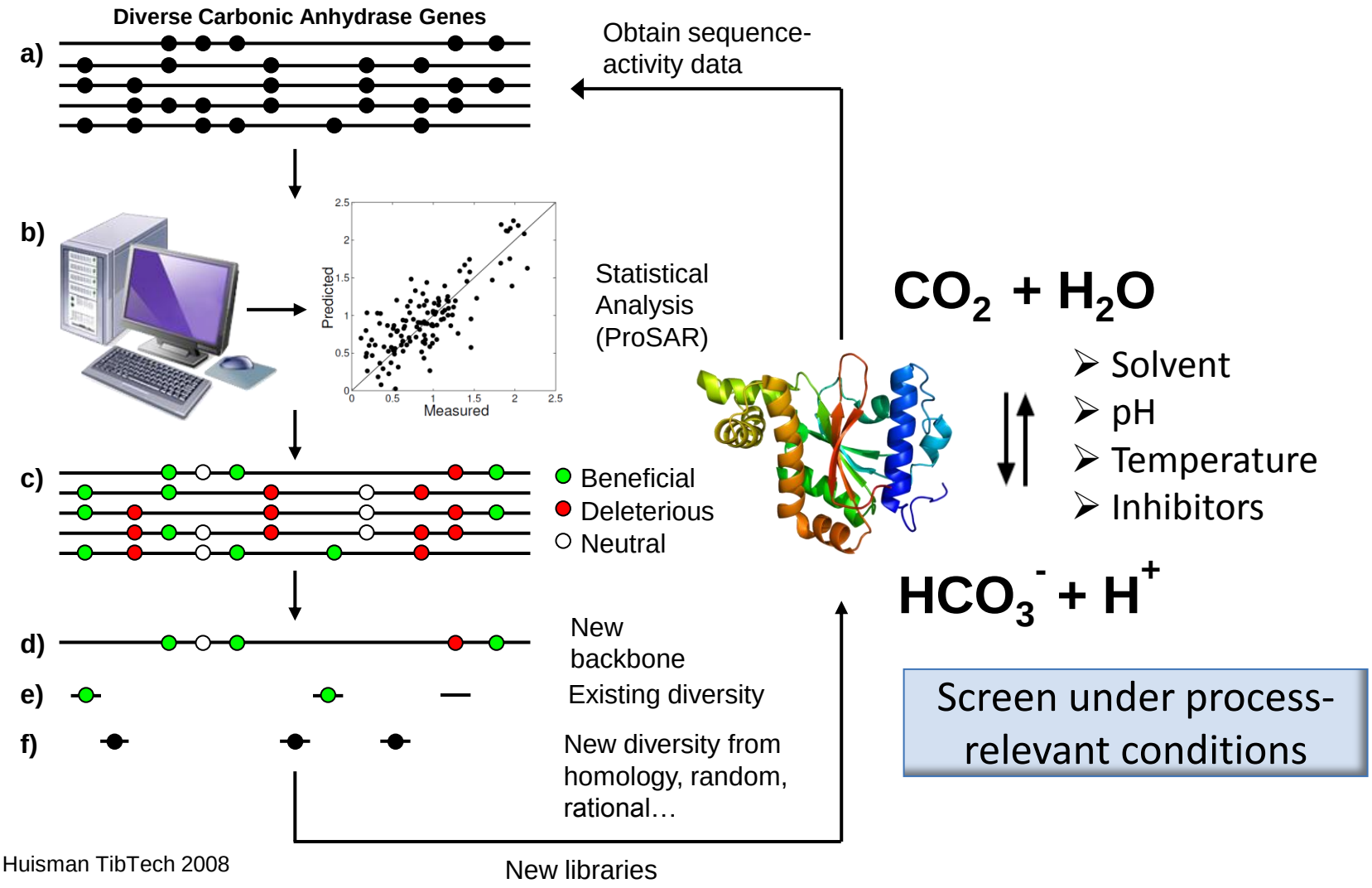
hCAII:

- Good activity, low stability.

CA-102:

- Accelerates CO_2 capture at modest concentrations (<1 g/L).
- Good thermostability.
- Natively secreted

Increasing the Stability of Carbonic Anhydrase



Fox & Huisman TibTech 2008

Evolving CA-102 for Solvent & Thermostability

A) Challenge enzymes 24h

B) Assay for remaining activity

C) Increase challenge toward process conditions round-by-round

4.2M MDEA, >120°C

Rd 6: 4.2M MDEA, 87°C

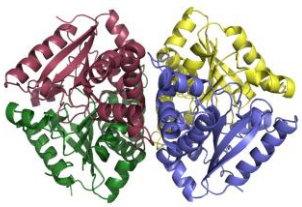
Rd 5: 4.2M MDEA, 85°C

Rd 4: 4.2M MDEA, 70°C

Rd 3: 4M MDEA, 65°C

Rd 2: 3M MDEA, 50°C

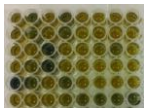
Rd 1: 3M MDEA, 42°C → Backbone $T_{20\%}^{24h}$



How do we screen our libraries?

Tier 1

− $CO_2 + H_2O \leftarrow HCO_3^- + H^+$
− low [amine] assay after 24h challenge
− monitor pH via absorbance


No MDEA


+ MDEA

Tier 2

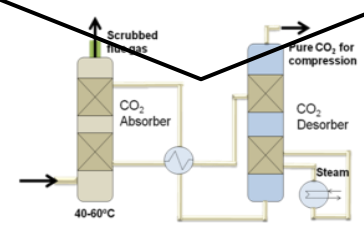
− $CO_2 + H_2O \leftarrow HC^{18}O_3^- + H^+$
+ ↑[amine]
+ monitor ^{18}O loss
+ process relevance

Tier 3

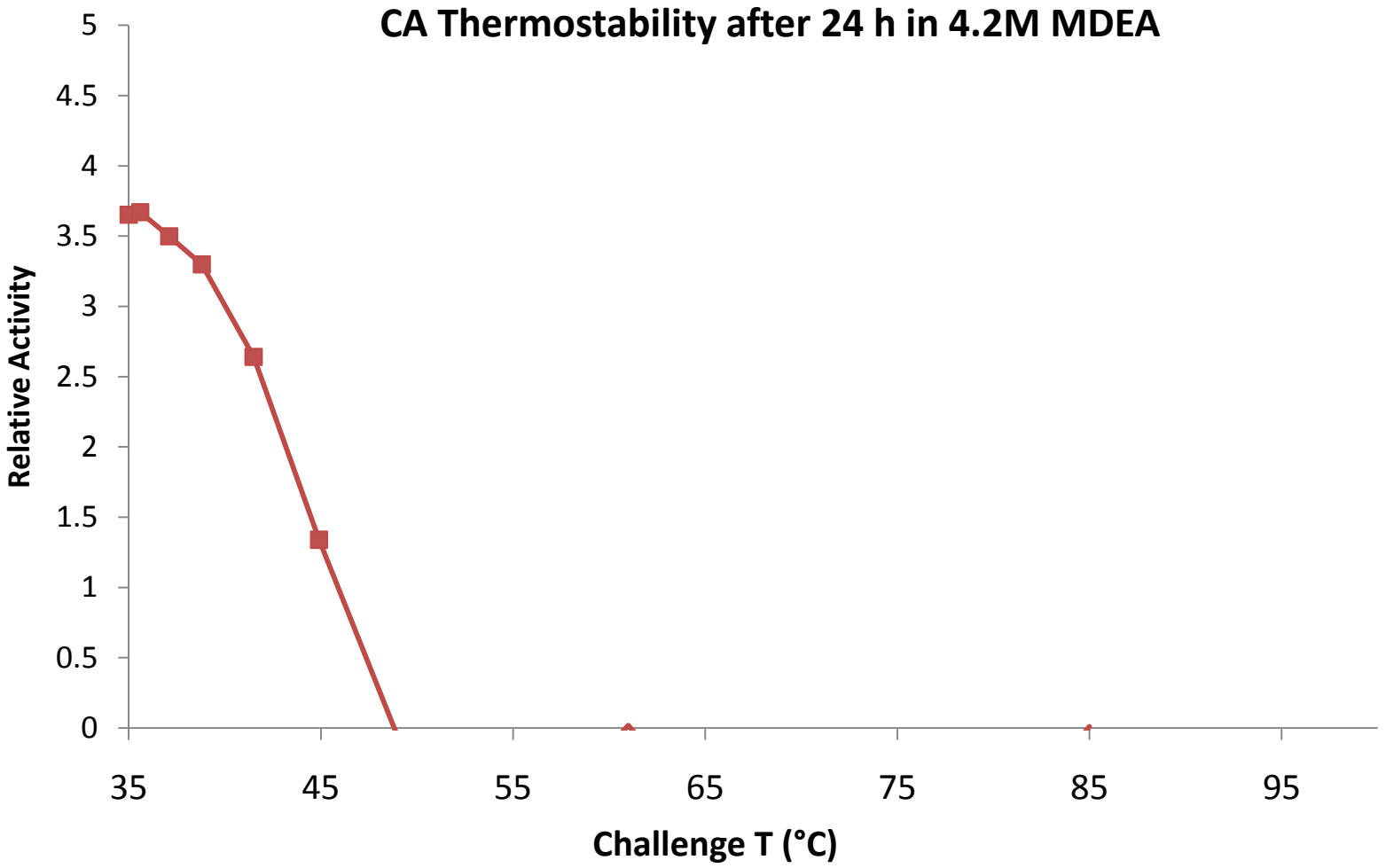
+ $CO_2 + H_2O \rightarrow HCO_3^- + H^+$
+ ↑[amine]
+ monitor CO_2 ΔP

Stirred Cell Reactor (SCR)

Pilot Plant

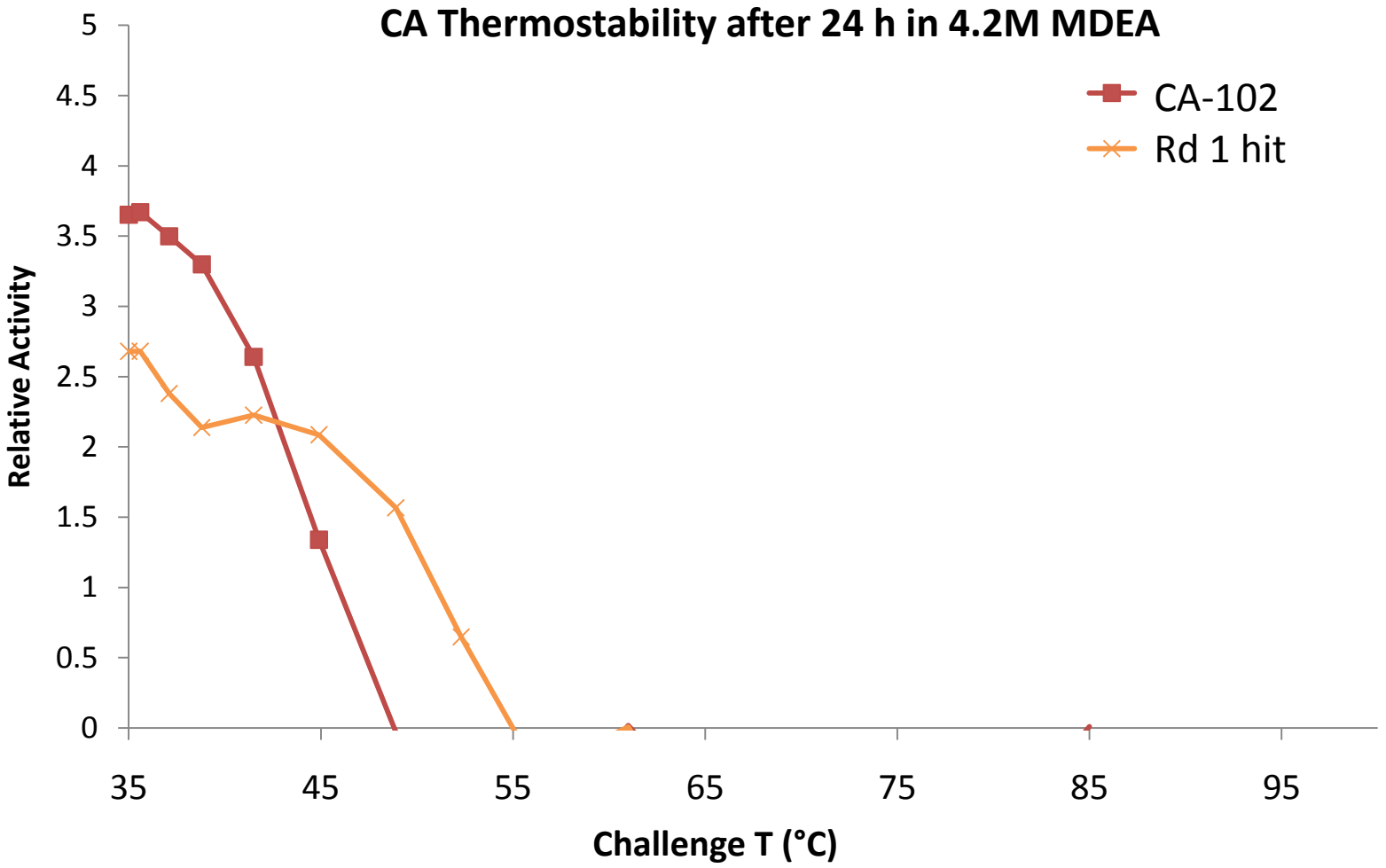


Inactivation Temperature in 4.2M MDEA after 24 h



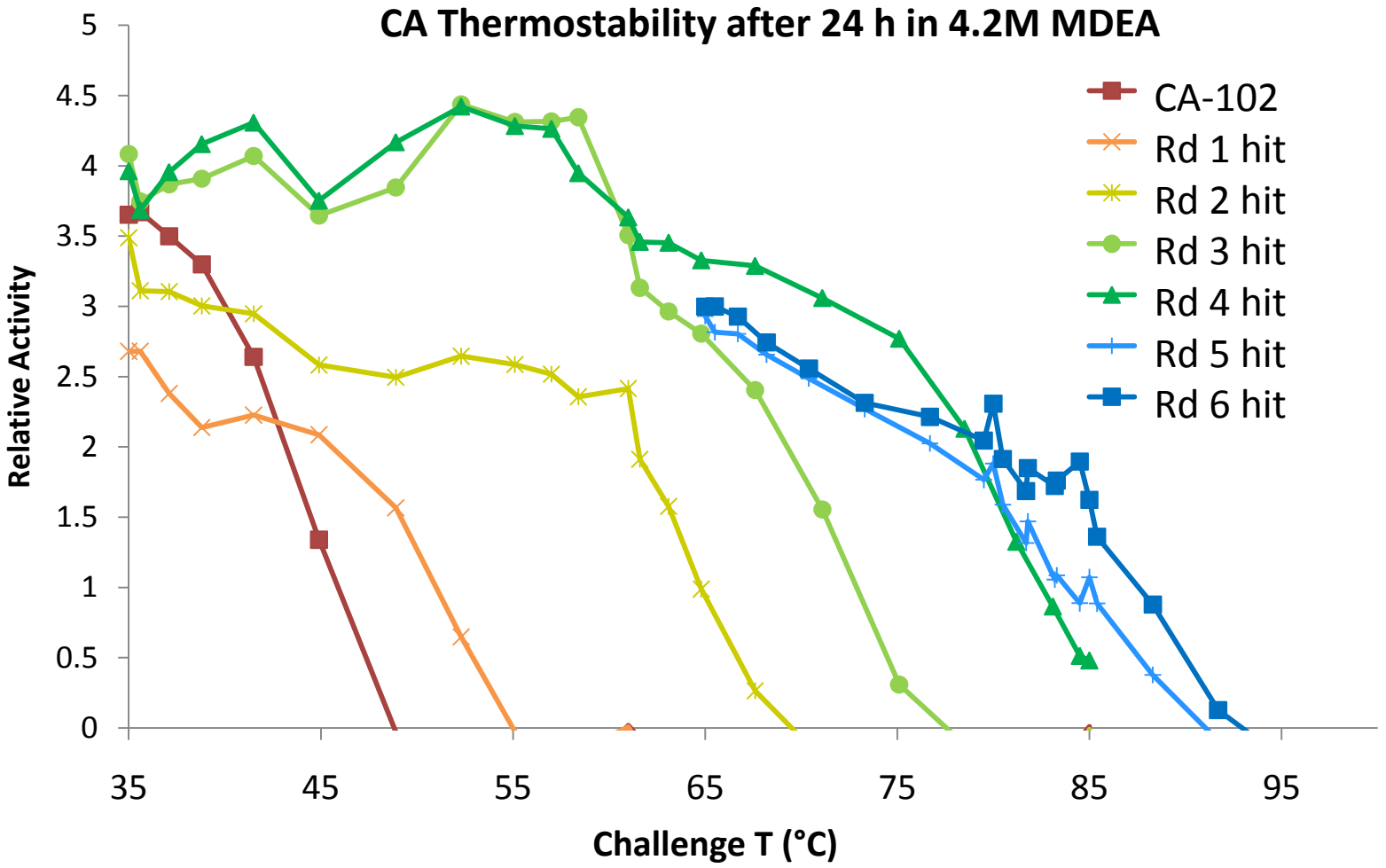
Best hits $T_{20\%}$: 45°C

Inactivation Temperature in 4.2M MDEA after 24 h



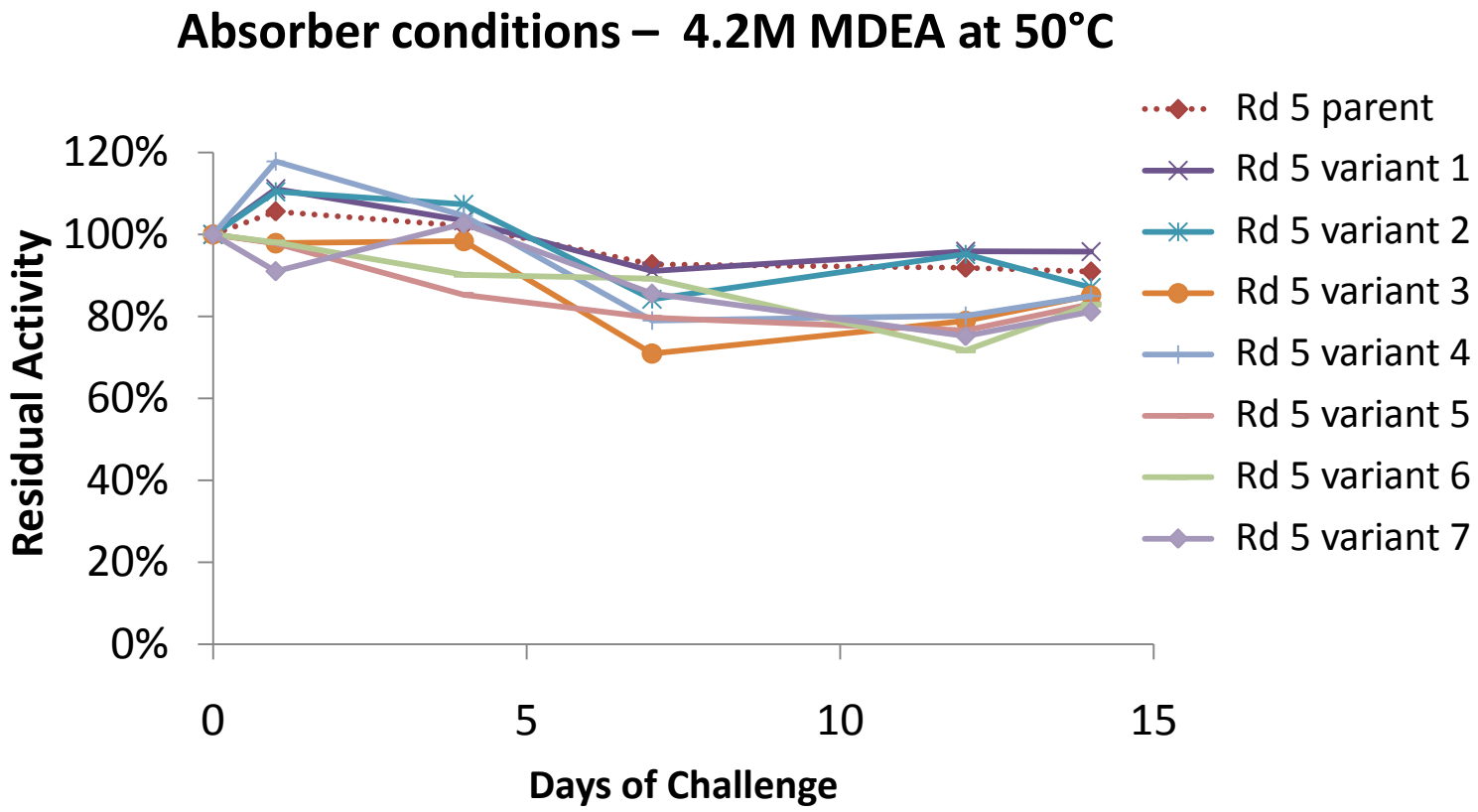
Best hits $T_{20\%}$: 45°C → 52°C

Inactivation Temperature in 4.2M MDEA after 24 h



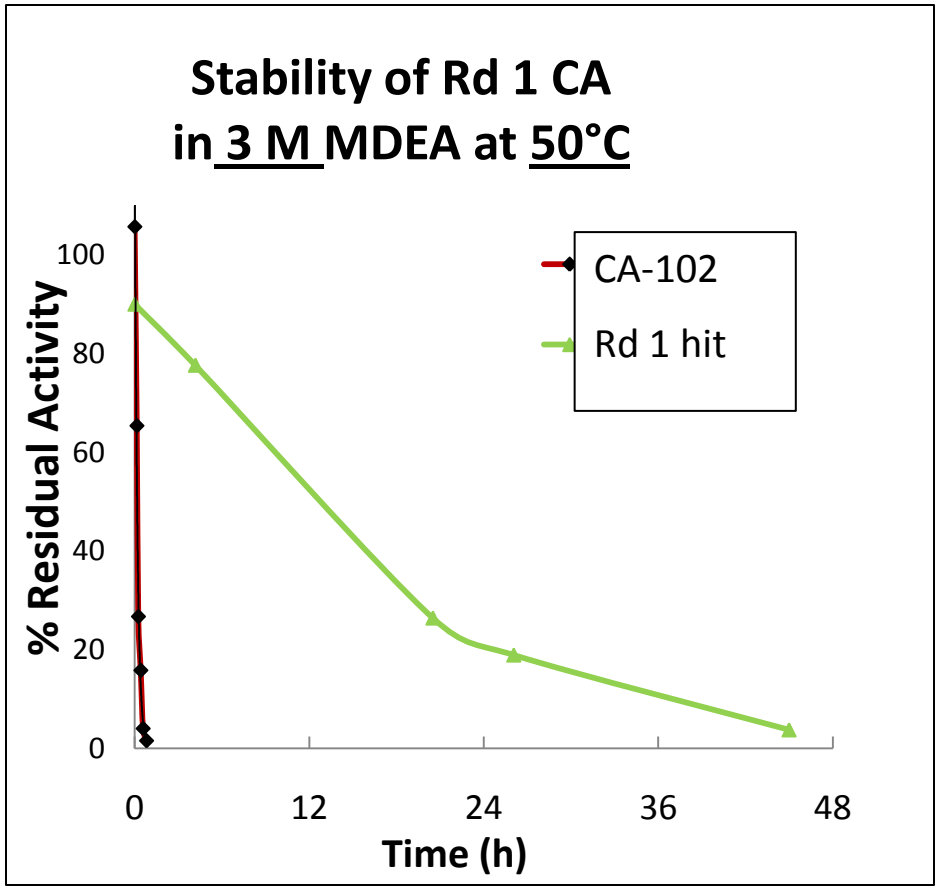
Best hits $T_{20\%}$: 45°C → 52°C → 65°C → 70°C → 85°C→ 87°→ 91°C

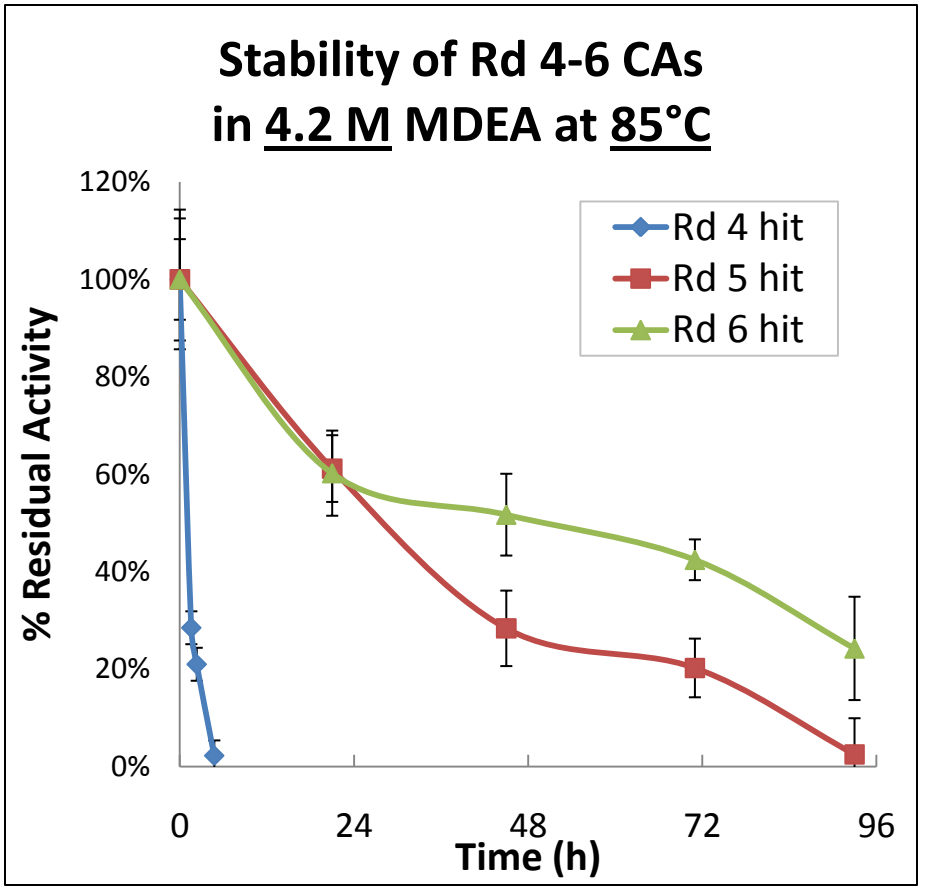
Long Term Stability



- 8 variants with > 80% residual activity on Day 14 (2 > 95%)

CA Stability at Desorber Conditions

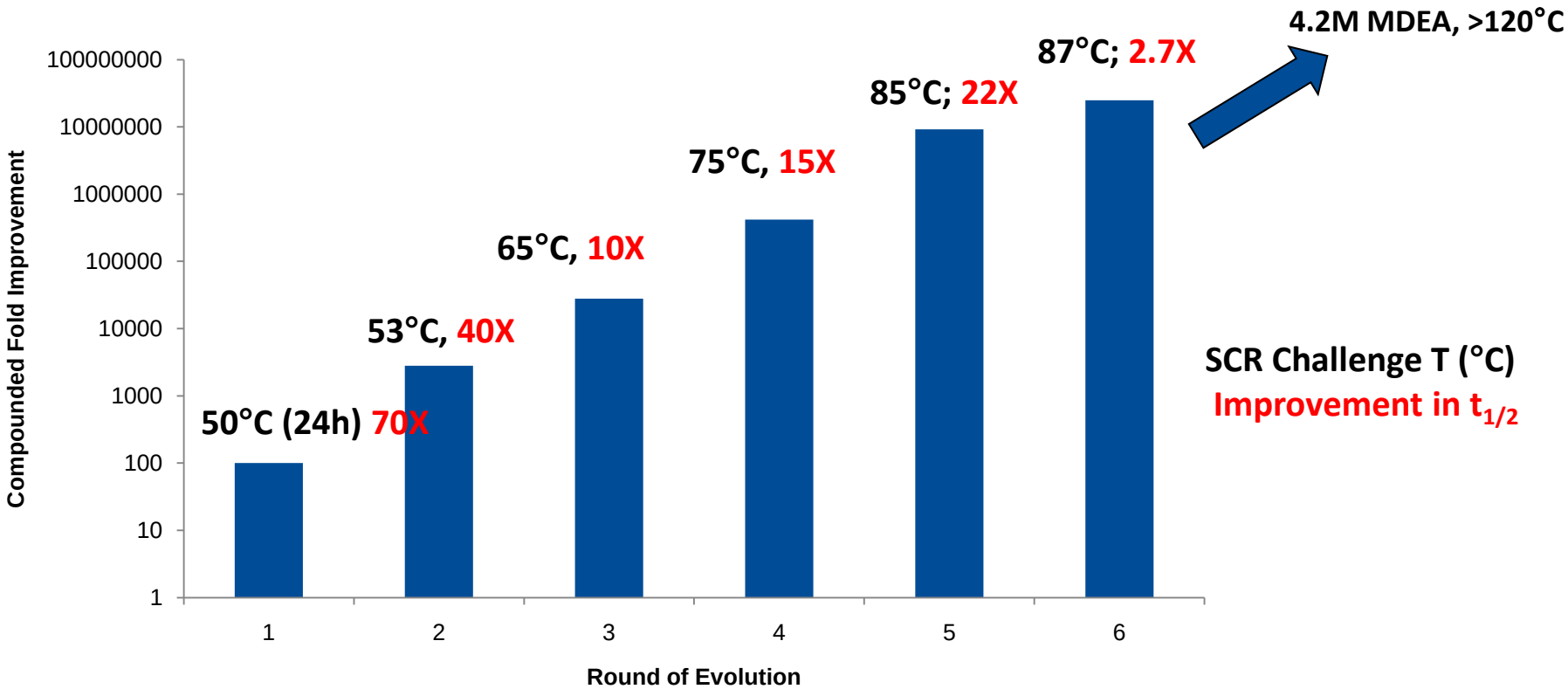




- CA-102: $t_{1/2}$ = 10 min
- Rd 1 hit: $t_{1/2}$ = 12 hrs

- Rd 4 hit: $t_{1/2}$ = 0.9 hr
- Rd 6 hit: $t_{1/2}$ = 52 hr

Directed Evolution of Biocatalyst for 4.2M MDEA



- 6 rounds of evolution increased thermostability (T_{50} after 24 h) by >50°C.
- Best variants: 90+% active after 2 weeks in absorber conditions (50°C, 4.2M MDEA)

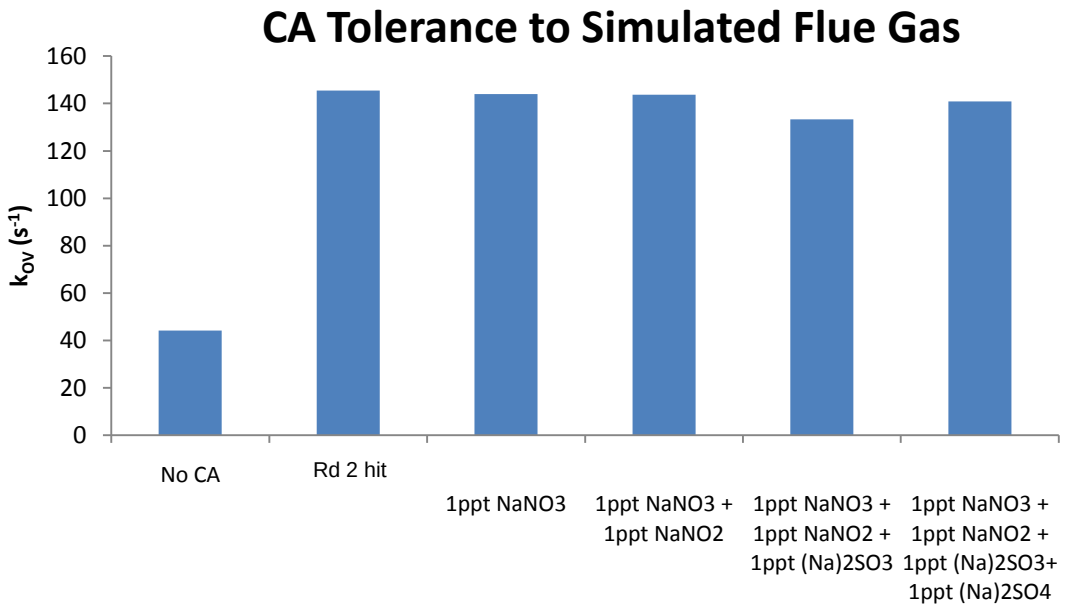
  25% active after 4 days at 85°C in 4.2M MDEA

  20% active after 1 day at 91°C in 4.2M MDEA

Tolerance to Flue Gas Components

Estimated minor flue gas components

Components	Concentration
SOx (ppm)	85
SO2 (ppm)	10
NO2 (ppm)	70
NOx (ppm)	500



Conditions: 4.2M MDEA (α = 0.02), 50°C;
1 ppt = parts per thousand

- No significant effect by any of the salts under these conditions.
- Long term effects are unknown.
- Planning bench-scale test at National Carbon Capture Center using authentic flue gas.

Current Deployment Projects

Power Generation

- Exclusive agreement with energy infrastructure and carbon capture technology company.
- Pilot testing of CO_2 Solution / Codexis technology for coal-fired power plants.
- Expandable to full commercialization upon pilot success.

Aluminum Industry

- Exclusive agreement with Alcoa, Inc., the world's leading producer of aluminum.
- $16.8 million multi-phase project funded by U.S. Department of Energy and Alcoa.
- CO_2 emissions are captured and combined with aluminum/other industry process residuals to produce commercial soil building product and 'green' fertilizer.
  - CO_2 is neutralized.
  - Industrial residuals beneficiated.
- Codexis/CO_2 Solution technology enables process.

ALCOA

CO_2 Solution

Summary: ARPA-E Year 1

- Six rounds of directed evolution have developed thermostable, MDEA-tolerant carbonic anhydrases
  - > 2 weeks @ 50°C (absorber conditions)
  - 24 hours @ 91°C (approaching desorber conditions)

- Evolved CA's are tolerant to flue gas components

Next:

- Continue evolution for tolerance to process conditions
- Test enzymes in real flue gas

Acknowledgement

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